In the Application of: John E. Sims and Blair R. Renshaw

Docket No.: 2932-B

Serial No.: 09/976,472

Group Art Unit: 1646

Filing Date: October 11, 2001

Examiner: J. Andres

For: IL-1 eta DNA and Polypeptides

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop AF
Commissioner for Patents
P. O. Box 1540
Alexandria, VA 22313-1450

Considered
1/12/04

Sir:

I, John E. Sims, do hereby declare as follows:

1. I am currently employed as a Distinguished Fellow in the Department of Molecular Immunology of Immunex Corporation, a wholly-owned subsidiary of Amgen Inc. I received a Ph.D. degree from Harvard University in Biochemistry; a copy of my curriculum vitae is attached. I am a co-inventor of the subject matter disclosed and claimed in the above-identified patent application.

2. As described in the attached Sims et al. article (*TRENDS in Immunology*, 22(10):536; 2001), since the time the original application was filed disclosing and claiming IL-1 eta polynucleotides and polypeptides, a new nomenclature has been implemented for the IL-1 family. Under that new nomenclature system, IL-1 eta is referred to as IL-1F8 (i.e., the eighth member of the IL-1 family). I use that nomenclature throughout this Declaration, but I affirm that IL-1 eta and IL-1F8 are the same protein.

3. I prepared or directed the preparation of an expression vector used to express IL-1 F8 as a glutathione S-transferase (GST) fusion protein in *E. coli*. The fusion protein was purified and used in several experiments to analyze its activity.

4. In one such experiment, NCI/ADR-RES cells were plated into 24-well dishes, and stimulated on the following day with IL-1F8. Supernatants were collected 48hrs after addition of the IL-1F8 and analyzed for the presence of various cytokines (IL-1beta, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12(p70), TNF-alpha, IFN-gamma and GM-CSF) using a commercially available cytokine detection system (the Beadlyte™ human multi-cytokine detection system 3 from Upstate Cell Signaling Solutions, Lake Placid, NY) as per manufacturer's instructions. Plates were read on a Luminex^{100} LabMAP ™ System (Luminex Corporation, Austin, TX) and results analyzed using Masterplex QT software (MiraBio Inc., Alameda, CA).